

REMARKS

Upon entry of this amendment, claims 1-20 are pending. Claims 1, 4, 10-12, 15, and 16 have been amended. Claims 18-20 has been added. Claim 11 has been canceled.

Support for the amendment to claims 1 and 4 is found at least at dependent claim 11 (incorporated therein and canceled) and ¶[0048] of the publication of the application, US Pub 2006/0127907 which states:

[0048] A specific condition under which the base sequence of the probe does not inhibit the DNA amplification includes a condition that the Tm of the probe is designed to be 25 to 40.degree. C. (preferably 30 to 35.degree. C.) lower than the Tm of primers.

Support for the amendment to claim 10 is found at least at claim 10. Support for the amendments to claim 12 is found at least at claim 1 and ¶[0048]. Support for the amendment to claim 15 is found at least at claim 15. Support for the amendment to claim 16 is found at least at claim 16.

Support for new claim 18 appears at least at ¶[0053]. Support for new claims 19-20 appears at least at claim 1, ¶[0049], and Table 2. Paragraphs [0049] and [0053] are reproduced below with emphasis added.

[0049] For example, with consideration given to general conditions of the PCR method, the probe should be typically 10-mers to 13-mers. This is much shorter than 15-mer to 25-mer probes (see the above-mentioned non-patent reference 2) that have been conventionally used as a probe for allele specific oligonucleotide hybridization. In the context of that longer probes have been extensively used heretofore, there has been the theory that a sequence of at least about the 15th power of 4 is required for constructing a probe having specificity by combinations of four different bases in the whole genome sequence (3 billion base pairs). However, this holds true for the case where hybridization is directed toward the whole genome sequences. When hybridization is directed to a PCR-amplified DNA fragment having several hundreds of bases, such length or specificity is not considered necessary for probes that the specificity of hybridization is sufficiently maintained with the former probe as shown above.

[0053] The use of a short length of the probe designed as described above provides the following three advantages: 1) the difference in Tm values between the case where there is a mismatch of a single base and the case where there is no mismatch can be rendered larger than that of a longer length of a probe, and thus the specificity of the probe can be relatively increased; 2) the hybridization temperature of the probe can be given as low as 25.degree. C. in the detection method of the present invention, although conventionally 37 to 65.degree. C., and thus a subsequent series of procedures can be carried out at room temperature; and 3) a short length of the probe has a reduced Tm value and does not hybridize during PCR reaction, and thus the probe does not affect the PCR reaction even though it is previously mixed in the PCR reaction solution. This probe enables the procedures PCR.fwdarw.heat denaturation.fwdarw.hybridization to be carried out as a series of reactions, without performance of additional procedures such as the addition of a reagent during the reactions. These advantages can be similarly obtained in other DNA amplification methods with the use of an extension reaction by DNA polymerase, as in the PCR method.

No new matter has been added by way of this response.

Withdrawn Rejections under 35 U.S.C. § 112, ¶2 : Second Paragraph

Applicants acknowledge and thank the Office for withdrawing the rejection of claims 1-3 under 35 U.S.C. §112 as being indefinite.

Withdrawn Rejections under 35 U.S.C. §103(a)

Applicants acknowledge and thank the Office for withdrawing the rejection of claims 1, 3, 4, and 6 under 35 U.S.C. §103 as being obvious over Lay et al. in view of Klepp et al.

Applicants acknowledge and thank the Office for withdrawing the rejection of claims 2 and 5 under 35 U.S.C. §103 as being obvious over Lay et al. in view of Klepp et al. and Gunneberg et al.

Claim Rejections under 35 U.S.C. § 112, ¶1 : New Matter

Applicants respectfully traverse and, for the following reasons, request reconsideration and withdrawal of the rejection of claims 10-12 and 16 under 35 U.S.C. §112, ¶1 as failing to comply with the written description requirement. The claims contain subject matter that was described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

First, the Office asserts that the present specification lacks support for hybridization of the probe not substantially occurring, as that phrase is recited in claims 10 and 16. While disagreeing with the Office's interpretation, in the interest of furthering prosecution, claims 10 and 16 have been amended so as to remove "substantially".

Second, the Office asserts that no specific support appears for "the at least one labeled primer" as recited in claims 11 and 12. In the interest of furthering prosecution, claims 11 and 12 have been amended so as to recite "primers used in the DNA amplification". Support for such term appears in claim 1 and ¶[0048].

New Claim Rejections under 35 U.S.C. § 112, ¶2 : Second Paragraph

Applicants respectfully traverse and, for the following reasons, request reconsideration and withdrawal of the rejection of claims 10 and 15 under 35 U.S.C. §112, ¶2 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

First, the Office asserts that claim 10 is indefinite with respect to recitation of "hybridization of the target sequence does not substantially occur". In the interest of furthering prosecution, claim 10 has been amended to remove the term "substantially."

Second, the Office asserts that claim 15 is indefinite with respect to recitation of "at about a middle of the hybridization probe". In the interest of furthering prosecution, claim 15 has been amended to remove the term "about."

Claim Rejections under 35 U.S.C. §103(a) over Link in view of Klepp and Fu

Applicants respectfully traverse and, for the following reasons, requests reconsideration and withdrawal of the rejection of claims 1, 3, 4, 6-12 and 16 under 35 U.S.C. §103(a) as being unpatentable over Link et al., U.S. Patent No: 5,635,347 ("Link"), in view of Klepp (2000) Biochemica 2:14-16 ("Klepp"), and further in view of Fu et al., U.S. Patent No: 6,583,112 ("Fu").

To establish obviousness of a claim, the prior art must disclose or suggest each element of the claim; there must be some reason that would have prompted one or ordinary skill in the art to combine the elements and/or modify a reference so as to reach the requirements of the claim; and there must have been a reasonable expectation of success of the combination and/or modification. MPEP § 2143; *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. __, Slip Op No. 04-1350, 119 Fed. Appx. 282 (April 30, 2007).

The present invention is directed to chromatographic detection of polynucleotide sequences having mutation sites via differentially labeled primers and hybridization probes. The hybridization probes are generally shorter than those conventionally used in the art for allele specific oligonucleotide hybridization (see ¶[0049]). The shorter length hybridization probe and the difference in T_m between primer and probes provides, for example, increased specificity, lower hybridization temperatures allowing room temperature subsequent procedures, and/or no hybridization during the DNA amplification reaction (see ¶[0053]). The chromatographic detection methods described in the present application are versatile, rapid (e.g., completed in minutes, rather than hours), and simple (e.g., macroscopic determination without need for specialized instruments) (see ¶[0064]).

Claim 1 is directed to a method of detecting a target base sequence. Claim 1 recites amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase; hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected; and detecting a hybrid formed by the hybridization, wherein at least one of primers to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent, the hybridization probe is labeled with a second labeling agent and contained in a reaction solution for effecting the DNA amplification, the hybridization probe has a T_m of 25°C to 40°C lower than the T_m of primers used in the DNA amplification; the base

sequence of the hybridization probe is designed not to inhibit the DNA amplification, and the hybrid is detected by affinity chromatography with the use of the first and second labeling agents.

Link does not teach or suggest all elements of claim 1

Link is directed to a method of detecting a nucleic acid sequence using immobilization labeled primers and electrochemiluminescent (ECL) labeled hybridization probes that requires a specialized ECL analyzer for detection of the hybridized amplified product/probe. Link teaches that optimal primer size is about 20-30 bases in length (the smallest primer used by Link was a 22mer) and that, as primer size decreases, the likelihood that the primer will non-specifically hybridize increases (see col. 2, ln. 12-17).

As acknowledged by the Office, Link fails to teach or suggest a labeled 10 - 15 mer hybridization probe. A prior art disclosure teaches away where it criticizes, discredits, or otherwise discourages the solution claimed (see MPEP §2141.02(VI)). **Because Link discourages use of smaller primers due to increases in non-specific hybridization**, Link teaches away from this solution (see MPEP § 2141.02(VI)).

As acknowledged by the Office, Link fails to teach or suggest affinity chromatography detection of the hybrid via the first and second labeling agents.

Furthermore, Link fails teach or suggest a hybridization probe with a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification. The Office asserts that the Tm of the hybridization probe must be *necessarily* lower than the Tm of the primers to prevent interference with the PCR reaction. But the Office fails to establish that the Tm of the hybridization probe is necessarily 25°C to 40°C lower than the Tm of primers. To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference. MPEP § 2112(IV). The fact that a certain result or characteristic *may* occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. MPEP § 2112(IV). Just because the Tm of the hybridization probe *may* be 25°C to 40°C lower than the Tm of primers does not establish inherency of that requirement. As such, the Office has failed to show how Link expressly or inherently provides for the Tm requirement of claim 1.

Klepp does not teach or suggest all elements of claim 1

Klepp is directed to a multi-step method to detect the success of a PCR amplification using a digoxigenic- or biotin-labeled PCR product in conjunction with chromatographic test strip detection. Klepp teaches amplification of a DNA molecule with a labeled primer followed by a separate hybridization reaction in which a labeled probe is added to an aliquot of the PCR reaction. But Klepp does not teach or suggest all the features of claim 1.

Klepp does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 1. In fact, Klepp teaches away (see MPEP § 2141.02(VI)) from such feature by requiring that the “size of the labeled oligonucleotide [hybridization probe] *must* range between 17 - 40 bases” (Klepp, p. 14, col. 2, ln. 7-8; emphasis added). The Office asserts that one cannot show nonobviousness by attacking references individually (see Action, p. 8). But under MPEP §2141.02(VI), a prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention. And a prior art disclosure teaches away where it criticizes, discredits, or otherwise discourages the solution claimed (see MPEP §2141.02(VI)). Because Klepp *discourages* use of a hybridization probe less than 17 bases in length (“*must* range between 17-40 bases”), and does not disclose any other alternative, Klepp teaches away from the requirement in claim 1 that the hybridization probe be a 10-15mer.

Furthermore, Klepp fails to teach or suggest a hybridization probe with a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification. As explained above, just because the Tm of the hybridization probe *may* be 25°C to 40°C lower than the Tm of primers does not establish inherency of that requirement. As such, the Office has failed to show how Klepp expressly or inherently provides for the Tm requirement of claim 1.

Fu does not teach or suggest all elements of claim 1

Fu is directed to nucleic acid molecules encoding Werner’s Syndrome gene products, expression vectors, and expression host cells. Fu describes DNA amplification where amplification using primers is separately conducted from detection using probes. Fu employs conventional methods of PCR amplification and detection of amplified products through hybridization to probes of at least 12 nucleotides and usually 14 to 18 nucleotides in length (see

e.g., col. 18, ln. 42-50). While Fu discloses that selection of probe length is “somewhat” dependent on use of the probe, and is within the skill in the art, Fu provides no guidance as to why a shorter probe might be desirable or uses for which a shorter probe would be desirable.

Fu does not teach or suggest all the features of claim 1. Fu does not teach or suggest labeling a primer with a first labeling agent. Fu does not teach or suggest a labeled hybridization contained in a DNA amplification reaction solution; rather, Fu discloses separate amplification and detection steps. Fu does not teach detection of the hybrid with both a first and a second labeling agent. And Fu does not teach detection of the hybrid affinity chromatography.

Furthermore, Fu fails to teach or suggest a hybridization probe with a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification. As explained above, just because the Tm of the hybridization probe *may* be 25°C to 40°C lower than the Tm of primers does not establish inherency of that requirement. As such, the Office has failed to show how Fu expressly or inherently provides for the Tm requirement of claim 1.

No reason provided to combine or modify cited references

Despite its obligation to do so, the Office has failed to provide a reason to modify the methods of Link, Klepp, or Fu so as to reach the requirements of claim 1. In a determination of obviousness, the proper question is whether one of ordinary skill in the art would have seen an obvious benefit to upgrading conventional methods of detecting PCR amplification products so as to reach the requirements of claim 1 (see *KSR Int'l Co.*, at 6). The mere fact that references can be combined or modified does not render the resultant combination obvious unless there is some reason that suggests the desirability of the combination. MPEP §2143.01(III).

The present application teaches that the combination of hybridization probes of 10-15mer size having a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification provides increased specificity and decreased hybridization temperature (e.g., allowing room temperature hybridization reactions); and provides for conducting a series of continual reactions without need for isolation/purification of intermediates and/or additional procedures or reagents.

The Supreme Court has stated that a need or problem addressed by the patent can provide a reason for combining elements in the manner claimed. *KSR Int'l Co.* at slip 5. But the

Office has failed to provide any need or problem, nor any resultant advantage, that would provide a reason to provide for 10-15mer labeled primers and a hybridization probe with a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification, as required by claim 1. The Office merely asserts that selection of short hybridization probes would be a “matter of design choice” but provides no rationale to support such a statement.

In rebuttal to the Office’s unsupported assertions, Applicants argue that the cited references describe no benefit from the use of shorter hybridization probes, and in fact, provide disincentives to combining or modifying the cited references so as to reach the requirements of claim 1. First, none of the cited references suggest any benefit of using shorter hybridization probes. Second, none of the cited references suggest using hybridization probes having a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification, much less any benefit thereof. And third, as described above, Link and Klepp *teach away* from using shorter hybridization probes in PCR amplification reactions containing both labeled primers and labeled hybridization probes. The only cited reference to disclose shorter hybridization probes, Fu, is limited to separate amplification and detection steps (*i.e.*, labeled hybridization probes are not in the amplification solution). As such, there is no apparent reason why one skilled in the art would desire to modify the PCR amplification reactions containing both labeled primers and labeled hybridization probes as described by Link and/or Klepp with a 10mer to 15mer labeled hybridization probe having a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification, especially in light of references teaching away from such modification.

In conclusion, because Link, Klepp, and Fu, either alone or in any known combination, fail to teach or suggest all required elements of claim 1, and the Office fails to provide sufficient reason to modify the cited references so as to reach the requirements of claim 1, such claim has not been shown to be obvious over Link in view of Klepp and Fu. The above argument applies equally to claim 1 and claims dependent thereon, such as claims 3 and 7-12. The above argument also applies equally to claim 4 (and claims dependent thereon, such as claim 6) to the extent such claims require, for example, hybridization probes of 10mer to 15mer size having a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification. The above argument also applies equally to claim 16 to the extent such claim require, for example, hybridization probes of 12mer to 13mer size having a Tm of 30°C to 35°C lower than the Tm of primers used in the DNA amplification.

Claim Rejections under 35 U.S.C. §103(a) over Link in view of Klepp, Fu, and Gunneberg

Applicants respectfully traverse and, for the following reasons, requests reconsideration and withdrawal of the rejection of claims 1, 2, 5, 15 and 17 under 35 U.S.C. §103(a) as being unpatentable over Link, Klepp, and Fu as above and further in view of Gunneberg et al. (1993) Clin. Chem. 39(10):2157-2162 ("Gunneberg").

First, Applicants request clarification of the status of claim 1 with respect to the rejection over Link in view of Klepp, Fu, and Gunneberg. The Office recites several features not present in claim 1 in support of the above rejection (e.g., unlabeled oligonucleotide to enhance the specificity of hybridization).

As described above, none of Link, Klepp, or Fu, either alone or in any known combination, teach or suggest all requirements of claim 1. Neither does Gunneberg.

Gunneberg is directed to a genotyping method with improved signal to noise ratio. Gunneberg describes the use of PCR amplification of the α -antitrypsin gene followed by membrane blotting sequential hybridization with a radiolabeled 21 mer hybridization probe and an unlabeled 21 mer competitive hybridization probe, washing of the membrane to remove unbound probes, and autoradiography used to detect the hybridized radiolabeled probe.

Gunneberg does not teach or suggest all requirements of claim 1. For example, Gunneberg does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 1. As another example, Gunneberg does not teach or suggest including a labeled hybridization probe in the DNA amplification reaction solution, as required by claim 1.

Not only does Gunneberg require separate amplification and hybridization reactions, Gunneberg further requires sequential hybridization of the unlabeled and labeled probes. In fact, Gunneberg arguably teaches away from joint hybridization with a labeled probe and an unlabeled competitive probe by citing problems with previous studies that attempted simultaneous hybridization (see Gunneberg, p. 2161, in ¶ bridging col. 1-2). The Office asserts simultaneous hybridization is an effective substitute for sequential hybridization in the methods

of Gunneberg. But sequential hybridization requires a host of other experimental design parameters, which are not necessarily compatible with the disclosed method of Gunneberg. Under MPEP §2144.03(A), it is not appropriate for the Office make unsupported assertions of scientific fact where such facts are not capable of instant and unquestionable demonstration. Here, the Office should support the assertion that simultaneous hybridization is interchangeable with sequential hybridization in the methods of Gunneberg by citation to a reference that provides such.

The above argument applies equally to claim 1 and claims dependent thereon, such as claims 2 and 15. The above argument also applies equally to claim 5 to the extent such claim requires, for example, hybridization probes of 10mer to 15mer size having a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification. The above argument also applies equally to claim 17 to the extent such claim require, for example, hybridization probes of 12mer to 13mer size having a Tm of 30°C to 35°C lower than the Tm of primers used in the DNA amplification.

CONCLUSION

Applicant respectfully requests withdrawal of the rejections and believes that the claims as presented represent allowable subject matter. If the Examiner desires, Applicant welcomes a telephone interview to expedite prosecution. Applicant believes there is no fee due at this time. However, the Commissioner is hereby authorized to deduct any deficiency or credit any overpayment with respect to this response to Deposit Account No. 19-3140.

Respectfully submitted,

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